

Patent Claims

1. A method for the detection of a target nucleic acid comprising the nucleic acid sequence of parvovirus B19 in a sample comprising the steps of
 - (a) providing a sample suspected to contain the target nucleic acid
 - 5 (b) providing a pair of primers comprising a first and a second primer whereby the first primer consists of at least contiguous 12 nucleotides of a nucleic acid sequence selected from the nucleic acid sequence SEQ ID NO: 2, and
10 whereby the second primer consists of at least contiguous 12 nucleotides of a nucleic acid sequence selected from the complementary sequence of the nucleic acid sequences SEQ ID NO: 3 or 4,
 - (c) amplifying the target nucleic acid,
 - (d) detecting the amplified target nucleic acid of step (c).
- 15 2. A method according to claim 1 wherein the first primer consists of at least 12 contiguous nucleotides of a nucleic acid sequence selected from the nucleic acid sequences SEQ ID NO: 6 or 7 and wherein the second primer consists of at least 12 contiguous nucleotides of the complementary sequence of a nucleic acid sequence selected from the nucleic acid sequences SEQ ID NO: 8 or 9.
- 20 3. A method according to claim 1 or 2 wherein the first primer has a nucleic acid sequence selected from the group of nucleic acid sequences SEQ ID NO: 12 to 15 and wherein the second primer has a nucleic acid sequence selected from the complementary sequence of the group of nucleic acid sequences SEQ ID NO: 16 to 17.
- 25 4. A method for the detection of a target nucleic acid comprising the nucleic acid sequence of parvovirus B19 in a sample comprising the steps of
 - (a) providing a sample suspected to contain the target nucleic acid,
 - (b) providing a pair of primers comprising a first and a second primer,
 - (c) amplifying the target nucleic acid,
 - 30 (d) contacting the sample with a probe under conditions for binding the probe to the target nucleic acid,

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- (e) detecting the binding product between the target nucleic acid and the probe as an indication of the presence of the target nucleic acid

characterized in that

- 5 the first primer consists of at least contiguous 12 nucleotides of a nucleic acid sequence selected from the nucleic acid sequence SEQ ID NO: 2, and whereby the second primer consists of at least contiguous 12 nucleotides of a nucleic acid sequence selected from the complementary sequence of the nucleic acid sequences SEQ ID NO: 3 or 4, and/or
- 10 the probe consists of at least 12 contiguous nucleotides of the nucleic acid sequence SEQ ID NO: 5 or a complementary sequence thereof.
5. The method according to claim 4 wherein the probe carries a label.
6. A method according to claim 5 wherein an additional probe carrying a label is contacted with the sample in step d) so that a pair of probes consisting of a
15 first and a second probe is contacted with the sample in step d).
7. A method according to claim 6 wherein said amplifying step c) comprises contacting the sample with the said pair of primers to produce an amplification product if the target nucleic acid is present in said sample, wherein said hybridizing step d) comprises contacting said sample with the
20 pair of probes, wherein the members of said pair of probes hybridize to said amplification product within no more than five nucleotides of each other, wherein the first probe of said pair of probes is labeled with a donor fluorescent label and wherein the second probe of said pair of probes is labeled with a corresponding acceptor fluorescent label;
- 25 and detecting the binding product between the target nucleic acid and the pair of probes in step e) by detecting the presence or absence of fluorescence resonance energy transfer between said donor fluorescent label of said first probe and said acceptor fluorescent label of said second probe, wherein the presence of fluorescence resonance energy transfer is indicative of the
30 presence of the target nucleic acid in the sample, and wherein the absence of

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fluorescence resonance energy transfer is indicative of the absence of the target nucleic acid in the sample.

8. The method according to claim 4 wherein the probe carries a first and a second label.
- 5 9. The method according to any of the claims 4 to 8, wherein the target nucleic acid in step c) is amplified with a template-dependent DNA polymerase.
- 10 10. The method according to any of the claims 8 to 9 whereby the binding product between the target nucleic acid and the probe in step (e) is detected by the quantity of the first or second fluorescent label that is released from the probe hybridized to the target nucleic acid by exonuclease hydrolysis by the template-dependent DNA polymerase.
11. A method according to any of the claims 4 to 10 wherein the probe consists of at least 12 contiguous nucleotides of the nucleic acid sequence SEQ ID NO: 10 or a complementary sequence thereof.
- 15 12. A method according to claim any of the claims 4 to 11 wherein the probe has the nucleic acid sequence SEQ ID NO: 11 or a complementary sequence thereof.
- 20 13. A method according to any of the claims 4 to 12 wherein the first primer consists of at least 12 contiguous nucleotides of a nucleic acid sequence selected from the nucleic acid sequences SEQ ID NO: 6 or 7 and wherein the second primer consists of at least 12 contiguous nucleotides of the complementary sequence of a nucleic acid sequence selected from the nucleic acid sequences SEQ ID NO: 8 or 9.
- 25 14. A method according to any of the claims 4 to 13 wherein the first primer has a nucleic acid sequence selected from the group of nucleic acid sequences SEQ ID NO: 12 to 15 and wherein the second primer has a nucleic acid sequence selected from the complementary sequence of the group of nucleic acid sequences SEQ ID NO: 16 to 17.

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15. A method according to any of the claims 4 to 14 wherein the primer and/ or the probe comprise a modified nucleotide or a non-nucleotide compound.
16. A method according to any of the claims 4 to 15 wherein other target nucleic acids are detected in the same reaction.
- 5 17. A method according to claim 16 wherein the other target nucleic acids comprise nucleic acid from hepatitis A virus, hepatitis B virus, hepatitis C virus, west nile virus or the human immunodeficiency virus.
- 10 18. An oligonucleotide whereby the nucleic acid sequence of the oligonucleotide is selected from the nucleic acid sequences SEQ ID NO: 12 to 15, from the nucleic acid sequences 10 or 11 or a complementary sequence thereof or from the complementary sequence of the nucleic acid sequences 16 or 17.
19. An oligonucleotide according to claim 19 which comprises a modified nucleotide or a non-nucleotide compound.
- 15 20. A pair of primers comprising a first and a second primer whereby the nucleic acid sequence of the first primer is selected from the nucleic acid sequences SEQ ID NO: 12 to 15 and whereby the nucleic acid sequence of the second primer is selected from the complementary sequence of the nucleic acid sequences 16 or 17.
- 20 21. Use of an oligonucleotide according to any of the claims 18 to 19 or a pair of primers according to claim 20 in a hybridisation reaction with a complementary nucleic acid.
22. Use of an oligonucleotide according to any of the claims 18 to 19 as a primer, probe or capture probe.
- 25 23. A kit comprising a template dependent DNA polymerase, nucleotides and an oligonucleotide according to any of the claims 18 to 19 or a pair of primers according to claim 20.

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